Alterations in Fine Structures of Myofibrils and Structural Proteins in Patients with Dilated Cardiomyopathy
—Studies with Biopsied Heart Tissues—

TAKASHI KATAGIRI, M.D., TORU KITSU, M.D., KAZUHIDE AKIYAMA, M.D.
YOUSHIKI TAKEYAMA, M.D., AND HIROKAZU NIITANI, M.D.

Ultrastructural and biochemical alterations in myofibrils (Mf) were studied in biopsied myocardial tissues in 11 patients with dilated cardiomyopathy (DCM) and compared with those in non-hypertrophic control and secondary hypertrophic (SHT) heart muscles.

Transverse diameters of biopsied cardiac myocytes increased significantly in both of SHT and DCM, and ultrastructural changes were similar in quality in both of them. Volume densities of Mf were 61.1 and 59.9% on average in control and SHT myocardial cells, respectively, and they were not significantly different. But in DCM volume density was significantly less (49.8% in left ventricular myocytes), and inverse relation between that and diameter of cardiac myocytes was observed (p < 0.01).

Electrophoretic pattern and relative composition of major structural proteins from control and SHT heart muscles were similar and statistically insignificant. In DCM, relative contents of myosin heavy chain and α-actinin decreased significantly and distinctly in all of cases suggesting primary degradation of myofibrils.

CLINICAL significance of dilated cardiomyopathy (DCM) lies mainly in the contractile failure due to atrophy and degradation of ventricular heart muscle, and the resulting refractory congestive heart failure makes the prognosis of the patients very unfavorable! The cause of such myocardial degeneration is still uncertain, however, the possibilities of viral infection, immune abnormality, and so forth have long been discussed as is being done in this symposium. And the common alterations in any type of DCM is the degeneration of ventricular heart muscle cells.

In the past many efforts have been made to find out the specific morphologic changes in cardiac myocytes in DCM, especially with the development of endomyocardial biopsy. Findings show that there are no DCM-specific morphologic changes which indicates that abnormal findings, observed in DCM, are all nonspecific and also observable in secondary cardiac hypertrophy (SHT) in such diseases as hypertensive, valvular and congenital heart diseases.

To clarify the DCM-specific myocardial changes, we performed a morphologic quantitative measurement of myofibrils (Mf) of cardiac myocytes by point count method. We also measured the quantitative microanalysis of the...
structural proteins which constitute Mf by crude extraction and SDS polyacrylamide gel electrophoresis in biopsied cardiac tissues in 11 patients with DCM.

PATIENTS AND METHODS

Myocardial Biopsy; Endomyocardial biopsy was carried out in 31 patients at the diagnostic cardiac catheterization utilizing a Machida type biopette. The constitution of the patients is as follows; 12 with non-hypertrophic control, i.e. with almost normal heart muscle, such as sick sinus syndrome, atrioventricular block, and neurocirculatory asthenia; 8 with SHT such as hypertensive and valvular heart diseases, and 11 with DCM. Permission for endomyocardial biopsy was obtained from all of the patients after explaining the value of the study. Biopsies were performed principally from the right ventricle (RV) in patients with non-hypertrophic control hearts, and from the ventricle of the overloaded side in SHT, i.e. in cases of hypertensive and aortic valvular heart diseases from the left ventricle (LV) and of mitral stenosis from RV. In 8 patients with DCM biopsy was done from both ventricles. Usually at least 3 pieces of endomyocardial tissue were biopsied for this study for the purpose of histologic and ultrastructural observations and for biochemical analysis of the structural proteins.

Electron Microscopic Morphometry; Four randomly selected tissue blocks from an endomyocardial specimen were immediately fixed in a solution containing 2% glutaraldehyde and 0.1 M Na cacodylate (pH 7.4) at 4°C for one hour, rinsed 3 times with 0.1 M Na cacodylate (pH 7.4) and immersed overnight in the same solution. They were dehydrated in a graded series of ethanols and propyrene oxide, embedded in Epon 812, and ultrathin-sectioned with a Porter-Blum MT2 ultramicrotome. Then they were double-stained with uranyl acetate and lead nitrate in an ordinary way, and observed at 3,000 magnifications with Hitachi HS-9 and H-300 electron microscopes. Five randomly selected electron micrographs from each block were enlarged at a final magnification of 7,500, and quantitative morphometry was carried out by the point-count method of Weibel and the co-workers. A mesh of 2 mm cross grid was set on an electron micrograph, and the numbers of spots of Mf, mitochondria (Mt) and other remaining sarcoplasm under the cross of grid were counted, as shown in Fig. 1. Therefore at least 20 cardiac myocytes were subjected to morphometric analysis in one patient, and total points and areas examined were 4,080 points and 46,400 μm².
TABLE I MORPHOMETRY OF BIOPSIED CARDIAC MYOCYTES AND COMPOSITIONS OF STRUCTURAL PROTEINS

<table>
<thead>
<tr>
<th></th>
<th>Control Hearts</th>
<th>Secondary Cardiac Hypertrophy</th>
<th>Dilated Cardiomyopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RV</td>
<td>LV</td>
<td>RV</td>
</tr>
<tr>
<td>Transverse Diameter (μm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.7 ± 1.1 (12)</td>
<td>19.5 ± 4.8** (8)</td>
<td>21.6 ± 6.7** (11)</td>
</tr>
<tr>
<td>Volume Density (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myofibrils</td>
<td>61.1 ± 0.4 (12)</td>
<td>59.9 ± 1.6 (8)</td>
<td>52.2 ± 2.1* (11)</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>23.0 ± 0.7 (12)</td>
<td>23.6 ± 1.9 (8)</td>
<td>18.9 ± 1.0* (11)</td>
</tr>
<tr>
<td>Remaining Sarcoplasm</td>
<td>15.9 ± 1.1 (12)</td>
<td>16.5 ± 1.8 (8)</td>
<td>28.9 ± 3.2* (11)</td>
</tr>
<tr>
<td>Myosin Heavy Chain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actin</td>
<td>1.26 ± 0.44 (6)</td>
<td>1.13 ± 0.11 (5)</td>
<td>0.48 ± 0.20*** (BV) (6)</td>
</tr>
<tr>
<td>α-Actinin</td>
<td>0.18 ± 0.03 (6)</td>
<td>0.18 ± 0.03 (5)</td>
<td>0.08 ± 0.04*** (BV) (6)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. Numbers in parentheses indicate the numbers of patients studied. *: p < 0.05, **: p < 0.01, and ***: p < 0.001 against values of the control heart muscle.

Preparation of the Structural Proteins; A piece of biopsied heart tissue weighed about 2.2 mg on the average with half of it being the endocardium. Therefore the wet weight of the myocardial tissue was about 1 mg per piece of myocardial tissue, as described in the previous paper. Briefly, a piece of heart tissue was immediately immersed in a solution containing 50% glycerol, 0.1 M NaCl, 0.005 M NaHCO₃ and 0.0001 M glycoetherdiaminetetraacetic acid (EGTA) at 4°C for 24 hours and at -20°C for night. The glycerinated heart tissue was homogenized with a Teflon-glass homogenizer and the soluble proteins were washed out together with glycine with the addition of 0.1 M NaCl, 0.005 M NaHCO₃ and 0.0001 M EGTA and the centrifugation at 5,000 × G for 10 min 3 times. With this procedure, the final precipitate was composed of fragmented myofibrils at greater than 95%. The final precipitate was reacted with Na dodecysulfate (SDS) by the direct addition of a solution containing SDS and β-mercaptoethanol, and the subunits of the structural proteins were separated by polyacrylamide gel electrophoresis with gels with 3 mm in diameter by the modified method of Weber and Osborn. Gels were stained with Coomassie Brilliant Blue and the composition of each protein was calculated from densitometric curves.

RESULTS

1. Morphometric Analysis

1) Transverse diameter of the biopsied cardiac myocytes.

Transverse diameter of the non-hypertrophic control human heart muscle cells ranged 12.7 ± 1.1 μm in RV, as shown in Table I. The normal ranges of transverse diameter of cardiac myocytes in our laboratory are roughly estimated to be 8–12 and 9–14 μm for RV and LV cells, respectively. In the cardiac myocytes of the overloaded ventricles of SHT hearts, the transverse diameters were 19.5 ± 4.8 μm, and were significantly larger than those of the control heart muscle cells (p < 0.01). Furthermore, cardiac myocytes from patients with DCM were 21.6 ± 4.2 μm with respect to RV and LV in transverse diameters, and were as large as those of the myocytes from SHT. And this observation was true in all of cardiac myocytes from patients with DCM.

2) Volume densities of myofibrils and mitochondria.

Volume densities of Mf and Mt of the biopsied cardiac myocytes are summarized in the upper part of Table I. The volume density of Mf was 61.1 ± 0.4% in the non-hypertrophic control cardiac myocytes and that of Mt was 23.0 ± 0.7. Therefore the remaining sarcoplasm of the cells occupied 15.9 ± 1.1%.

In SHT myocardial cells, the volume density of Mf and Mt were 59.9 and 23.6% on the average, respectively, and these were not significantly different from those of the control heart in t statistics. In myocardial cells from patients with DCM, on the other hand, the volume densities of Mf and Mt distributed to 52.2 and
Fig. 2. Volume density of myofibrils and transverse diameter of biopsied cardiac myocytes. Significant inverse relation is noted between volume density of myofibrils and transverse diameter in left ventricular cells in patients with dilated cardiomyopathy. ○: control heart, ×: secondary hypertrophic heart, ◦: right, and ■: left ventricular cells in dilated cardiomyopathy.

Fig. 3. Volume density of myofibrils of biopsied cardiac myocytes and left ventricular enddiastolic volume index (LVEDVI). Inverse correlation of volume density and LVEDVI is noted in left ventricular myocytes. ○: right, and ×: left ventricular myocytes. 
\[ r = -0.84, p < 0.01. \]

18.9% in RV, and 49.8 and 17.6% in LV, respectively, and were statistically different from those of the control and SHT heart muscle cells (p < 0.05). Namely, the volume densities of Mf and Mt were less in DCM.

*Japanese Circulation Journal Vol. 31, June 1987*
Fig. 4. Electrophoretic gels of the crude structural proteins from biopsied heart muscle. 10% acrylamide. SSS; sick sinus syndrome, NCA; neurocirculatory asthenia, HHD; hypertensive heart disease, Als; aortic insufficiency and stenosis, and MS; mitral stenosis.

Fig. 5. Electrophoretic gels of crude structural proteins from biopsied myocardial tissues in patients with dilated cardiomyopathy (DCM). 10% acrylamide.

Fig. 2, indicating the constancy of Mf content. In SHT hearts, it is situated in the similar high level as that of the control heart muscle cells, even though their transverse diameter increased. This indicates that the volume density of Mf increased in proportion to an increase in transverse diameter of the cardiac myocytes.

In cardiac myocytes from patients with DCM, volume density of Mf decreased in contrast to that from SHT heart muscle cells, notwithstanding an increase in the transverse diameter, in almost all of the myocytes obtained by endomyocardial biopsy. A statistically significant inverse interrelation was also observed between the volume density and the transverse diameter in LV cells (p < 0.05).

Figure 3 shows left ventricular enddiastolic volume index (LVEDVI) and the volume density
of the cardiac myocytes in DCM. Similar to Fig. 2, volume density of Mf decreased in proportion to an increase in LVEDVI, and an inverse relationship was also noted between the volume density and LVEDVI in LV myocytes (p < 0.01).

2. Structural Proteins

Electrophoretic gels of the structural proteins from the biopsied human cardiac tissues are shown in Figs. 4 and 5. In the control human myocardium, the pattern of the structural protein subunits was similar to those of the calf and canine hearts, as described previously. The structural proteins were separated according to molecular weights from the top of a gel, to myosin heavy chain (approximately 210,000 daltons), α-actinin (88,000), actin (46,000), tropomyosin (36,000), and another smaller sized proteins such as troponin subunits and myosin light chains. In SHT, the pattern of the structural proteins was similar to that of the control hearts (Fig. 4). In contrast, the pattern of the structural proteins in DCM was characteristic. Reductions in the bands of myosin heavy chain and α-actinin were noted in all of the cases and the stainability of the protein bands was less because of the smaller content of the structural proteins as shown in Fig. 5. In addition, the smaller subunits than troponin-T were hardly identified or, in cases of being identified, hardly determined by densitometry. The molecular weights of the structural proteins were not different from those of the control hearts. In some patients whose volume density of Mf was less, the bands of the structural proteins were faint, and were difficult to determine.

Myosin heavy chain, α-actinin and actin were seen in all DCM cases, and their compositions were presented in the lower part of Table I as expressed to the ratio of the content of actin. In the non-hypertrophic control heart, average myosin heavy chain/actin and α-actinin/actin were 1.26 and 0.18, respectively. In SHT hearts, they were 1.11 and 0.18, respectively, and statistically not different from those of the control hearts. In DCM, reductions in the relative contents of myosin heavy chain and α-actinin were shown clearly. The average of myosin heavy chain and α-actinin to actin were 0.48 and 0.08, respectively, and they were significantly less than those of the control and SHT hearts in t-statistics (p < 0.001).

DISCUSSION

The poor prognosis of patients with DCM is attributable in most part to the refractory heart failure which originates in reduced contractility. Development of endomyocardial biopsy through cardiac catheter by Sakakibara and Konno has made it possible to study the living heart tissue from patients with various heart diseases including cardiomyopathies. Since then many studies concerning cardiomyopathies, mainly histopathologic ones, have been carried out and a considerable amount of myocellular abnormal findings have been reported such as disarray and degenerations of myofibrils, mitochondrial swelling and disruption, nuclear deformation and so forth. However, these findings have proven to be nonspecific from the histopathologic standpoint to DCM, but observable in another heart diseases such as SHT, i.e. congenital and valvular heart diseases. Also several works have been carried out on the biochemical viewpoint utilizing biopsied myocellular specimens such as enzymic activities of the whole myocardial homogenate, but their results are still controversial.

In this study we attempted to find out the DCM-specific myocardial alterations utilizing tiny biopsied cardiac specimens in the combination of ultrastructural and biochemical measures from the standpoint of myofibrillar changes which are mostly responsible for the lowered myocardial contractility in DCM.

Even though the morphologic findings are non-specific to DCM in quality, the quantitative morphologic data presented in this paper brought new evidence. That is, myocellular hypertrophy occurs both in DCM and SHT, but the volume density became less in the former in proportion to an increase in myocellular transverse diameter. Meanwhile it increased with an increment of the diameter in the latter. This phenomenon indicates that even though myocellular hypertrophy is initiated in compensation for reduced contractility presumably due to cellular degradation, an increase in myofibrils may not be attained, but is inversely degraded in DCM. This was the most extraordinary fact obtained by quantitative morphometry. Schwartz and the co-workers first studied biopsied heart tissues from a large number of patients with DCM by quantitative morphometric analysis under a light microscope, and they observed the decrease in the volume fraction of Mf in correlation of left ventricular
dysfunction.\textsuperscript{10} In addition to a decrease in the volume density of MF, reductions in myocardial structural proteins were also noted in biochemical analysis. As details have already been described previously,\textsuperscript{5} the relative contents of myosin and actin, which are the major components of thick filament and z-band, respectively, decreased distinctively in all of the cases of DCM. On the contrary in heart muscle from SHT, the compositions of the structural proteins remained at the same level as the control hearts.

These morphologic and biochemical facts on myofibrillar changes indicate that the hypertrophic style in DCM is different from that in SHT. That is to say that degradation of myofibrils or contractile proteins originates primarily in DCM, and loss of myofibrils and resulting cell death initiate refractory contractile failure. A similar changing pattern of the structural proteins was observed in acute myocardial infarction and also in the skeletal muscle of Duchenne type progressive muscular dystrophy.\textsuperscript{5} These facts lead to the suggestion that degradations in myosin and actin indicate the primary degradation of the structural proteins in DCM.

Because the coronary circulation is well maintained in DCM, many acidic reactions that play a role in myocardial necrosis in the ischemic state\textsuperscript{11} may not be attributable to the pathogenesis of myocellular degradation in DCM. But reactions following the excess inflow of Ca\textsuperscript{2+} into cardiac myocytes\textsuperscript{12} such as the activations of Ca\textsuperscript{2+}-activated neutral proteases and phospholipases A\textsubscript{2} and C are conceivable, and these should be initiated by the impairment of sarcolemmal permeability, even though such sarcolemmal changes have not been proven in DCM.

The amount of biopsied myocardial tissue is so tiny that the biochemical analysis of membrane-composing microorgans has scarcely been carried out, and these fields of studies should be proceeded by new techniaues such as freeze fracture electron microscopy and lanthanum probe method in the future.

Acknowledgement

The authors wish to express sincere thanks to Professor Yasumitsu Nakai of Showa University School of Medicine for his kind guidance to the technique of electron microscopy. They are also greatly indebted to Drs Youichi Kobayashi, Sumiyasu Sekita, Hitoshi Kanaya, Yutaka Tabata, and Fumihiro Tanno for their collaboration.

REFERENCES